

# The Survival of Smallpox Virus (*Variola Minor*) in Natural Circumstances

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The introduction of smallpox virus into communities normally free from the disease can in most cases be traced to the arrival of persons from regions that are still subject to smallpox outbreaks. Failure occasionally to discover the means of introduction of infection has led to considerable speculation. Cotton was suspected in England of being a vehicle for the transport of the disease to Lancashire from abroad in outbreaks between 1908 and 1952.<sup>a</sup> MacCallum & McDonald<sup>b</sup> studied the survival of smallpox virus in scabs under various circumstances. They came to the conclusion "that a few particles of virus may survive for as long as 18 months", and stated that the "virus can survive for many years, ten or more, at from 4°C to 5°C in closed tubes or bottles, with little decrease in titre". Such conditions, however, will normally not be encountered.

The problem of survival of smallpox virus under natural circumstances is of the utmost importance at the present time. A WHO-sponsored world-wide programme of smallpox eradication is under way, and the question arises whether there is a need to be greatly concerned about the possibility of human infection originating from old scabs or other virus residues present in a country. This problem is more complex than that involved in the mere demonstration of some viable virus particles; such particles must be virulent and must be present in numbers large enough to cause infection.

In early March 1954, during a variola minor epidemic<sup>c, d</sup> we collected a large number of scabs from 3 patients. All scabs from each patient were placed in a separate, unsealed envelope, and all these envelopes were in turn placed in a single large envelope, also unsealed. We used these scabs to compare different techniques of virus isolation and diagnosis, but as this investigation did not yield any new or important results the work was abandoned, and the large envelope containing scabs was stored in a cupboard in the laboratory. Some years later we inves-

tigated the viability of the smallpox virus in those scabs. We found that we were able to isolate variola minor virus, and since that time we have tried to isolate the virus from these scabs nearly every year.

## Materials and methods

In winter, the temperature of the laboratory in which the cupboard containing the envelope with scabs was situated was kept above 20°C in the daytime, but might drop as low as 15°C at night; during week-ends the temperatures were slightly lower. In summer, temperatures as high as 30°C were noted on some occasions, but, again, the night temperature would be 15°C or sometimes even lower. The relative humidity in the laboratory varied from 35% to 98%. As an egg-incubator was situated in the laboratory, air saturated with water vapour at a temperature of 39°C was blown into the room. This egg-incubator, however, was used at irregular intervals.

Six scabs (2 from each envelope) were put into a mortar and ground together with 10 ml of bacteriological broth containing 10 IU penicillin G and 0.25 mg dihydrostreptomycin per ml. (Dry scabs were not ground as this was found to cause a fine dust that could be smelled outside the inoculation cabinet. Indeed, merely opening the envelopes to take out the scabs (inside the cabinet) gave rise to a typical dusty smell in the laboratory.)

The scab-broth mixture was centrifuged for 20 minutes at 3000 rev/min in an angle centrifuge. The supernatant was then diluted in a tenfold series. Aliquots of 0.1 ml of each dilution were then inoculated into the chorioallantoic membrane of each of 5 eggs. 0.1 ml of the most concentrated fluid contained the material of about 1/20th of a scab; dilution 2 contained material from 1/200th of a scab; dilution 3 material from 1/2000th of a scab, and so on. The eggs were incubated at 39.4°C for 8 days before the chorioallantoic membrane was dropped and inoculated. They were then reincubated for another 72 hours at a temperature of 35°C.<sup>e, f</sup>

<sup>a</sup> Dixon, C. W. (1962) *Smallpox*, London, Churchill, pp. 303-305.

<sup>b</sup> MacCallum, F. O. & McDonald, J. R. (1957) *Bull. Wild Hlth Org.*, **16**, 247-254.

<sup>c</sup> Jong, M. de (1955) *Acta Leidensia*, **25**, 142-232.

<sup>d</sup> Blomhert, G. (1956) *Docum. Med. geogr. trop. (Amst.)*, **8**, 197.

<sup>e</sup> Dinger, J. E. (1956) *Docum. Med. geogr. trop. (Amst.)*, **8**, 202.

<sup>f</sup> Kempe, C. H. (1956) *Variola and vaccinia*. In: American Public Health Association, *Diagnostic procedures for virus and rickettsial diseases*, 2nd ed., New York, pp. 341-361.

## Results

The results of our titrations over the years may be summarized as follows (these figures are calculated by the method of Reed & Muench<sup>g</sup> from the real values<sup>h</sup>):

Year	Interval	No. of viable particles per scab
1954	0	Scabs obtained (no titration available)
1956	2 years	56 000
1957	3 years	52 000
1958	4 years	24 000
1959	5 years	32 000
1960	6 years	12 000
1961	7 years	3 600
1962	8 years	1 250
1963	9 years	1 500
1964	10 years	1 700
1964	nearly 11 years	80
1967	13 years	490
1967*	13 years	2 900*

\* Slightly different procedure.

In 1956, about 2½ years after the scabs had been collected, 56 000 viable particles per scab were demonstrated. Four years later (1960) 1/5th of this amount could be isolated; a further 4 years later the amount was 1/7th of that in 1960 and 1/33rd of that in 1956.

In 1967, 490 particles per scab could still be demonstrated, and the investigation was repeated using a slightly different procedure: 10-day-old eggs instead of 8-day-old eggs were inoculated. This yielded 2900 viable particles per scab. The differences in the number of particles demonstrated in the experiments may be due in part to the variation in quantity of virus present in the scabs.

In all experiments the virus could be subinoculated on to other chorioallantoic membranes, giving rise to typical smallpox lesions confirmed by the histological and serological examinations. The temperature-sensitivity and the late deaths of the embryos also showed clearly that the isolated strains were of variola minor (alastrim).

## Discussion

Under certain natural environmental conditions, it is evident, as shown in these studies, that variola virus may survive for long periods in scabs. During the 1953-54 alastrim outbreak in The Hague, scabs must have played an important role as infecting agents.<sup>c,i</sup> At that time, the scabs contained at least 50 000 viable particles and perhaps 100 000 or more. With the passage of time, the number of particles detected has decreased steadily. Whether or not, under natural circumstances, persons could become infected after contact with this scab material is not known.

Europe, during the past 20 years, has been free of endemic smallpox. Outbreaks, when they have occurred, have almost always been traced to infection introduced from endemic areas. The origins of a few outbreaks, however, have remained obscure. It is conceivable that some of these may have originated through infection of individuals coming in contact with scabs present in old clothes, rags or books. If infection has occurred in this manner, it must be regarded as a rare event indeed considering the very substantial non-immune or only partially immune population which undoubtedly has had chance contact on many occasions with scab material.

Further information regarding the possible importance of old scabs as sources of infection should be sought through careful investigation of all outbreaks of smallpox occurring in smallpox-free areas of the world.

<sup>g</sup> Reed, L. J. & Muench, H. (1938) *Amer. J. Hyg.*, **27**, 493.

<sup>h</sup> Gispen, R. (1953) *Antonie v. Leeuwenhoek*, **19**, 149.

<sup>i</sup> Jong, M. de (1956) *Docum. Med. geogr. trop. (Amst.)*, **8**, 207.

## Transliteration from Cyrillic characters

The "International System for the Transliteration of Cyrillic Characters", set out in Recommendation ISO/R9-1954 (E) of the International Organization for Standardization, is normally used in the *Bulletin of the World Health Organization* for personal names, titles of publications, etc. However, papers accepted for publication may contain names transliterated differently, and if the original Cyrillic spelling is not recognizable inconsistencies may occur.

For convenience the transliteration from Russian according to ISO/R9 is given below:

## Translittération des Caractères cyrilliques

Le «Système international pour la translittération des caractères cyrilliques» présenté dans la Recommandation ISO/R9-1954 (F) de l'Organisation internationale de Normalisation est généralement utilisé dans le *Bulletin de l'Organisation mondiale de la Santé* pour les noms de personnes, les titres de publications, etc. Cependant des articles acceptés pour publication peuvent contenir des noms translittérés différemment et si l'orthographe cyrillique originale n'est pas reconnaissable un manque d'uniformité peut s'ensuivre.

A toutes fins utiles, la translittération du russe selon la recommandation ISO/R9 est indiquée ci-après:

Cyrillic character Caractère cyrillique	Trans- literation from Russian Trans- littération du russe	Examples and remarks Exemples et observations	Cyrillic character Caractère cyrillique	Trans- literation from Russian Trans- littération du russe	Examples and remarks Exemples et observations
А, а	a	Адрес = Adres	У, у	u	Утро = Utro
Б, б	b	Баба = Baba	Ф, ф	f	Физика = Fizika
В, в	v	Вы = Vy	Х, х	h	Химический = Himičeskij
Г, г	g	Глава = Glava Голова = Golova	Ц, ц	c	Центральный = Central'nyj
Д, д	d	Да = Da	Ч, ч	č	Часы = Časy
Е, е (ё) <sup>1</sup>	e (ë)	Ещё = Eščë	Ш, ш	š	Школа = Škola
Ж, ж	ž	Журнал = Žurnal	Щ, щ	šč	Щека = Ščeka
З, з	z	Звезда = Zvezda	(medial, médial)  Ъ, ъ  (final)	"or" "ou"  (Not trans- literated. Non trans- littéré.)	In modern Russian, where ' sometimes replaces medial ъ, transliteration is still " En russe moderne, où le ' remplace quelquefois le ъ médial, la translittération reste "
И, и	i	Или = Ili			
Й, й	j	-ый, -ий, -ой = -yj, -ij, -oj			
К, к	k	Как = Kak			
Л, л	l	Любить = Ljubit'			
М, м	m	Муж = Muž			
Н, н	n	Нижний = Nižnij			
О, о	o	Общество = Obščestvo			
П, п	p	Первый = Pervyj	Ы, ы	y	Был = Byl
Р, р	r	Рыба = Ryba	Ь, ь	'or' 'ou'	Маленький = Malen'kij
С, с	s	Сестра = Sestra	Э, э	ě	Это = Ěto
Т, т	t	Товарищ = Tovarišč	Ю, ю	ju	Южный = Južnyj
			Я, я	ja	Яйцо = Jajco

<sup>1</sup> Cyrillic ё to be transliterated by ě only when the diacritical appears in the original. Le ё cyrillique ne doit être translittéré par ě que lorsque la diacritique apparaît dans l'original.